VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The paragraph starting on page 16, line 12, has been amended as follows:

--Figure 16 (SEQ ID NOS: 11-13, and 18-21) shows a schematic for β-globin genotyping. A 214 bp fragment of the b-globin gene is illustrated. The sense and anti-sense DNA strands are shown with exon 1 centrally located. Arrows indicate forward and reverse primers. Mutation probes are indicated as Probe 1 (LC Red 640 labeled) for codon 6 and Probe 2 (LC Red 705 labeled) for codon 26. The dual-labeled fluorescein probe is positioned between Probes 1 and 2. Point mutations are indicated by bold capitalized script and duplex mismatches by subscripts.--

The paragraph starting on page 17, line 1, has been amended as follows:

--Figure 18 (SEQ ID NOS:22-25) shows a schematic of probes to investigate one of the hyper-variable regions in HLA-A, at codons 62-67 in exon 2. Variable bases are indicated in bold type. Just upstream of this region is an area that is mostly conserved among the various HLA-A alleles. We will synthesize one fluorescein donor probe and two acceptor probes, one labeled with LCRed640 and one with LCRed705.--

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The paragraph starting on page 17, line 10, has been amended as follows:

--Figure 20 (SEQ ID NOS:26-30) shows a schematic of probes for investigating variation within the HLA-DRB1 region by color and Tm multiplexing. Codons 70-74 of exon 2 are hyper-variable and adjacent to a conserved region.--

The section starting on page 35, line 18, with the heading Table I., has been amended as follows:

--Table I. Oligonucleotides and Probes Used for Genotyping the Model Apolipoprotein E Locus

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Sequences for Genotyping Codon 112

ε3 Target GGCGCAGGCCCGGCTGGGCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGT [a] (SEQ ID NO: 1)

ε4 Target GGCGCAGGCCCGGCTGGGCGCGGACATGGAGGACGTGCGCGGCCGCCTGGTGCAGT [a] (SEQ ID NO: 2)

Fluorescent Probes CCAGGCGGCCGCACACG-fluorescein (SEQ ID NO: 3)

LC Red 705-CCTCCATGTCCGCGCCCAGCCGGGCCTGCG (SEQ ID NO: 4)

Sequences for Genotyping Codon 158

ε2 Target GCGGCTCCTGCCCGATGCCGATGACCTGCAGAAGTGCCTGGCCAGTGTACCA [a] (SEQ ID NO: 5)

ε3 Target GCGGCTCCTGCCCGATGCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCA [a] (SEQ ID NO: 6)

Fluorescent Probes ACACTGCCAGGCACTTC-fluorescein (SEQ ID NO: 7)

LC Red 640-GCAGGTCATCGGCATCGGGCAGGAGCC (SEQ ID NO: 8)

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[a] Underlined area indicates fluorescein probe target region.--

The paragraph starting on page 43, line 7, has been amended as follows:

--*Materials and Methods*

The human b-globin gene sequence (GenBank Accession U01317) was used to design primers and probes for the amplification of a 214 bp segment containing exon 1 (Figure 16). Due to high homology between b-globin and d-globin sequences, the primers (sense: GTCAGGGCAGAGCCATCTA (SEQ ID NO: 9), antisense: GTTCTATTGGTCTCCTTAAAGGTG, SEQ ID NO: 10) were designed with 3' and additional mismatches to d-globin. Due to the close proximity of the hemoglobin mutations, a unique combination of probes were designed to detect Hb S, C, and E alleles. Two probe and LightCycler Red 705 (LC Red 705, Roche Molecular Biochemicals, Indianapolis, IN), as mutation detection probes. The third probe was a dual-labeled fluorescein donor probe which spans the distance between the mutation detection probes. When annealed, resonance energy is transferred from each fluorescein label to either the LC Red 640 or the LC Red 705 labeled probes. The codon 6 detection probe (CTCCTGTGGAGAAGTCTGC- LC Red 640, SEQ ID NO: 11) completely matched the Hb S allele anti-sense strand. The codon 26 probe (LCR 705-GTTGGTGGTAAGGCCCTGG-phosphate SEQ ID NO: 12) completely matched the Hb E allele anti-sense strand. Both the LC Red 640 and LC Red 705 probes were obtained

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from Idaho Technology Biochem (Salt Lake City, UT). The fluorescein-labeled probe was labeled with two fluoresceins (F) attached to the 5' and 3' ends (F-GTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGA-F, SEQ ID NO: 13) (Operon, Alameda, CA). Fifty-five blinded samples of human genomic DNA were randomly selected from samples submitted to Neo Gen Screening for sickle cell hemoglobinopathy screening. The DNA (80-130 ng) was prepared from blots on filter paper and had been previously genotyped by allele specific cleavage and gel electrophoresis.--

The section starting on page 45, line 27, has been amended as follows:

--One of the hyper-variable regions in HLA-A is at codons 62-67 in exon 2 (Fig. 18). Variable bases are indicated in bold type. Just upstream of this region is an area that is mostly conserved among the various HLA-A alleles. One fluorescein donor probe and two acceptor probes, one labeled with LCRed640 and one with LCRed705, are synthesized. (Lay MJ et al., *Clin. Chem.* 43:2262-2267 (1997); Bernard et al., *Am. J. Path.* 153:1055-1061 (1998); Bernard et al., *Anal. Biochem.* 273:221-228 (1999)). A 182 bp region flanking the probes will be amplified by rapid cycle PCR with primers GACAGCGACGCCGCGAGC (SEQ ID NO: 14) and GGGCCGGGGTCACTCACCG (SEQ ID NO: 15). These primers have 3'-mismatches with all Class I loci except for HLA-A, which provides for allele specific amplification (Wittwer et al., *Clin. Chem.* 39:804-809 (1993)). The probes are included in the amplification mixture, PCR is performed and a melting curve is obtained at 0.1-0.2°C/sec.--

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The paragraph starting on page 46, line9, has been amended as follows:

--The acceptor probes are designed to melt between 50-73°C with all sequence groups (Fig. 19 and Table III). The donor probe serves as an anchor and remains annealed during the melting of the acceptor probes. Two-hundred previously-typed DNA samples (400 alleles) are tested (samples courtesy of Dr. Tom Fuller, consultant). With only the LCRed640 probe, it is possible to distinguish all 8 HLA-A sequence groups. Heterozygotes in sequence groups that melt near each other are the most difficult to distinguish. The smallest predicted Tm difference is 1.3°C between groups A and B. The addition of the LCRed705 probe unambiguously distinguishes all sequence groups. The probe Tms most useful in distinguishing between sequence groups are shown in bold type. The mean and variance of the actual Tms are compared to predicted values. Any disagreement between prior typing and LightCycler analysis are resolved by sequencing and repeat analysis.

Table III. Predicted Tms for 2 Probes at a HLA-A Variable Region

Sequence Group	HLA-A Alleles	Frequency (%)	Predicted Probe Tm LCRed 640	LCRed 705
A	0101-0103 0106 3601	15.2	71.6	63.3
B	3002-3006 3101 3103 3104 3201-3204 7401-7404	6.2	70.3	67.6
C	0301 0302 0304 0305	13.4	67.8	65.0
D	0201 0202-0207 0209-0219 24XX 16 additional 02XX	27.2	66.1	72.3
E	2301-2305 2402-2406 8 additional 24XX	11.1	61.6	62.5

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F	2901-2903 7 additional 68XX	3.6	58.9	59.6	
G	2501 2503 2601-2606 2608-2612 3301-3305	7.2		56.8	57.8
H	2502 2613 6601 6602 6801-6802	~7		52.8	54.1

Total Estimated Frequency 90.9 –

The paragraph starting on page 46, line 22, has been amended as follows:

--In a similar fashion, variation within the HLA-DRB1 region is assessed by color and Tm multiplexing. Codons 70-74 of exon 2 are hyper-variable and adjacent to a conserved region (Fig. 20). One donor probe and 3 acceptor probes are synthesized. A 193 bp region flanking the probes are amplified with primers AGCGGGTGCGGTTCCTGG (SEQ ID NO: 16) and CAACCCCGTAGTTGTGTCTGCAGTAG (SEQ ID NO: 17). At least one of these primers is 3'-mismatched with all other DRB subclasses. It has been verified by sequencing that these primers specifically amplify only DRB1 alleles.--

The paragraph starting on page 47, line1, has been amended as follows:

--The acceptor probes are designed to melt under 70°C (Fig. 21 and Table IV). Melting temperatures below 40°C are difficult to obtain on the LightCycler and are not used for differentiation. The donor probe is stabilized with a minor groove binder to increase its Tm (Kutyavin et al., *Nucl. Acids Res.* 28:655-661 (2000)). The same 200 previously typed samples are analyzed. Most of the sequence groups are identified with the LCRed640- and Cy5-labeled

probes, although the LCRed705 probe is required for two groups. The smallest predicted Tm difference is 1.5°C between groups I and J. Any disagreement between prior typing and LightCycler analysis is resolved by sequencing and repeat analysis. The smallest Tm difference that is reliably distinguished in heterozygotes is determined. This establishes the maximum number of sequences that can be distinguished by Tm multiplexing.

Table IV. Predicted Tms for 2 Probes at a HLA DRB1 Variable Region

Sequence Group	DRB1 Alleles	Frequency (%)	Predicted Probe Tm LCRed 640	Cy5	LCRed 705
A	0101 0404 0405 0408 0410 1402 1406	15.8	69.5	59.0	23.8
B	0801 0802 0803 0804	4.1	61.2	45.7	28.3
C	1501 1502 1503	13.6	60.9	50.4	27.8
D	0401	6.4	60.1	43.6	35.3
E	0901 1401 1404	3.8	57.1	50.6	23.5
F	1001	0.9	54.5	46.9	<20.0
G	11011 11012 11014 1305 16012 16022	7.5	54.1	68.1	24.1
H	0103 0402 1102 1103 1301 1302 1304	13.1	46.4	59.0	26.1
I	1303	1.2	37.4	58.2	36.3
J	11013 1201 1202 16011 16021	4.5	35.5	56.7	<20.0
K	0301 0302 0303 0305	10.1	34.4	<20.0	59.2
L	0403 0407	2.0	29.3	53.6	33.6
M	0701	8.2	<20.0	46.9	58.0

Total Estimated Frequency 92.0 --

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On page 49, immediately preceding the claims, the enclosed text entitled "SEQUENCE LISTING" was inserted into the text.

Replacement pages for Figures 1, 8, 10-13, 18-21, clean versions and versions with markings to show changes made are attached.

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